12:03:42 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 02/11/91

Active

Project #: G-33-G15 Cost share #: Rev #: 3
Center # : 10/24-6-Q5169-5A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 GM18894-20 Mod #: ADMIN 2/11/91 Document : GRANT
Prime #: Contract entity: GIT

Subprojects ? : N
Main project #:

Project unit: CHEMISTRY Unit code: 02.010.136
Project director(s):
YU N-T CHEMISTRY (404)894-4007

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 900901 to 910831 (performance) 911130 (reports)

| Sponsor amount | New this change | Total to date |
|---|---|---|
| Contract value | 0.00 | 179,540.00 |
| Funded | 0.00 | 179,540.00 |
| Cost sharing amount | | 0.00 |

Does subcontracting plan apply ?: N

Title: LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. HELEN SUNSHINE
(301)496-7309

NATL INST OF GENERAL MEDICAL SCIENCE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

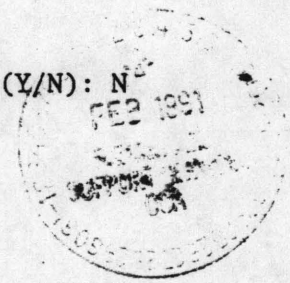
Sponsor issuing office

ANN CALURE
(301)496-7166

NATL INST OF GENERAL MEDICAL SCIENCE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

Security class (U,C,S,TS) : U
Defense priority rating : N/A
Equipment title vests with: Sponsor

ONR resident rep. is ACO (Y/N): N
NIH supplemental sheet
GIT X



Administrative comments -
ISSUED TO REVISE DELIVERABLE SCHEDULE.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 10/21/91

Project No. G-33-G15_____ Center No. 10/24-6-Q5169-5A0_

Project Director YU N-T_____ School/Lab CHEMISTRY_____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____

Contract/Grant No. 5 R01 GM18894-20_____ Contract Entity GIT_

Prime Contract No. _____

Title LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS_____

Effective Completion Date 910831 (Performance) 911130 (Reports)

| Closeout Actions Required: | Y/N | Date Submitted |
|---|---|---|
| Final Invoice or Copy of Final Invoice | N | _____ |
| Final Report of Inventions and/or Subcontracts | N | _____ |
| Government Property Inventory & Related Certificate | N | _____ |
| Classified Material Certificate | N | _____ |
| Release and Assignment | N | _____ |
| Other _____ | N | _____ |

Comments**CONTINUED BY G-33-G16** NO FINAL CLOSING DOCUMENTS ARE REQUIRED.___

Subproject Under Main Project No. _____

Continues Project No. G-33-G14_____

Distribution Required:

| | |
|---|---|
| Project Director | Y |
| Administrative Network Representative | Y |
| GTRI Accounting/Grants and Contracts | Y |
| Procurement/Supply Services | Y |
| Research Property Managment | Y |
| Research Security Services | N |
| Reports Coordinator (OCA) | N |
| GTRC | N |
| Project File | Y |
| Other _____ | N |
| _____ | N |

| SECTION IV<br>PROGRESS REPORT SUMMARY | GRANT NUMBER<br>GM18894-21 | |
|---|---|---|
| PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR<br>Yu, Nai-Teng | PERIOD COVERED BY THIS REPORT<br>FROM | THROUGH |
| APPLICANT ORGANIZATION<br>Georgia Institute of Technology | 09/01/90 | 06/12/91 |

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*

Laser-excited Raman Spectroscopy of Biopolymers

*(SEE INSTRUCTIONS)*

## 1. The Plans for the Next Year of Support:

The specific aims for the next year of support are : (1) To interpret the very unusual nature of the ligand binding site in liver fluke Dd hemoglobin, which gives rise to unusually low ν(Fe-CO) and unusually high ν(Fe-NO) frequencies; (2) To continue the development of *near IR-excited FT-Raman*, and *near IR-excited FT surface-enhanced Raman* technique; (2) To complete a paper on NO binding to model porphyrins; (3) To conclude our on-going studies on NO and azide binding to human hemoglobin mutants (*via* protein engineering; from Dr. K. Nagai); (4) To probe the effects of distal H-bonding on ligand vibrations of synthetic porphyrins with functional groups; (5) To elucidate the mechanism of the allosteric control of ligand binding to dimeric insect hemoglobins (samples from Professor K. Gersonde); (6) To analyze the resonance Raman data from hemoglobin M Iwate in term of the influence of tertiary structure on ligand binding (in β-subunits) within the frozen T-quaternary structure of tetrameric human hemoglobin.

## 2. Concise Description of the Studies Conducted during the Current Budget Year:

### a) *Resonance Raman studies of NO Binding to Protein-Free $Fe^{II}$ and $Fe^{III}$ Porphyrins*

With Soret-excited resonance Raman spectroscopy (RRS), we have detected the ν($Fe^{II}$-NO) and ν($Fe^{III}$-NO) stretching vibrations for several iron porphyrins, including octaethylporphyrin (OEP), tetraphenylporphyrin (TPP), heme-5, SP-13, SP-14, and SP-15. This result enables us to make the first comparison between RR spectra of nitrosyl complexes of iron porphyrins and hemoproteins, which is of interest if the

protein's effect on the Fe-NO bond is to be assessed. To investigate the dependence of the strength of the Fe-NO bond on electronic properties of the trans ligand, complexes of N-methylimidazole, pyridine, and tetrahydrofuran were prepared. We also compared complexes with sterically unhindered (N-methylimidazole) and hindered (1,2-Dimethylimidazole) proximal bases. Surprisingly, the n(Fe-NO) stretching frequency was found to be virtually insensitive to alterations in either electronic or steric properties of the trans-base.

b) ***Ligand Binding to Liver Fluke Dd Hemoglobin***

With excitation at 413 nm, we have identified the Fe(II)-NO, Fe(II)-CO and Fe(II)-$O_2$ stretching vibrations of the liver fluke Dd hemoglobin at 567, 571 and 478 $cm^{-1}$, respectively. Compared to the Fe(II)-NO stretching mode of nitrosyl myoglobin (Mb) and hemoglobin (Hb A) at 554 and 551 $cm^{-1}$, the $\nu(Fe^{II}\text{-}NO)$ frequency for Dd Hb.NO is ~15 $cm^{-1}$ higher, indicative of an unusual bonding geometry of NO. On the other hand, the $Fe^{II}$-CO stretch of Dd Hb.CO is ~32 $cm^{-1}$ lower than that of Mb.CO (507 $cm^{-1}$) and HbA.CO (512 $cm^{-1}$). The replacement of distal histidine by tyrosine in Dd Hb indeed has a profound effect on the $\nu(Fe^{II}\text{-}NO)$ frequency. Interestinly, such a replacement virtually has no effect on $\nu(Fe^{II}\text{-}O_2)$ frequency.

c) ***Surface-Enhanced Near-Infrared FT-Raman Scattering of Tetrathiafulvalene Adsorbed on Ag Powder***

We have demonstrated the use of an efficient and convenient silver substrate in the development of surface-enhanced near-IR FT-Raman scattering as a useful analytical technique that compliments the bulk FT-Raman method. The

SERS-active material, prepared in the form of solid silver powder by means of chemical precipitation, is particularly well-suited for surface Raman enhancement in the near-infrared region. To demonstrate the unique features of surface-enhanced FT-Raman, we also made a detailed spectroscopic study of the charge transfer donor molecule tetrathiafulvalene (TTF) adborbed on the silver powder. The advantages of near-IR FT-SERS compared with bulk FT-Raman include: (1) ability to analyze dilute solution samples, (2) acquisition of Raman spectra for near-IR fluorescent compounds, and (3) new vibrational information concerning the mixed-valence behavior of surface-bound TTF molecules.

3. No change

4. Not Applicable

5. **Publications:**


i) Nie, S., Marzilli, P. A., Marzilli, L. G. and Yu, N.-T. (**1990**) Near Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt $B_{12}$ and Model Compounds. Identification of the Co-C Bond Stretch in Cobalamins. ***J. Chem. Soc. Chem. Commun.*** 770-771.

ii) Nie, S., Marzilli, P.A., Marzilli, L. G. and Yu, N.-T. (**1990**) Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt $B_{12}$ and Model Compounds. 3. Vibrational Assessment of Factors Affecting the Co-C Bonds in Models. ***J. Am. Chem. Soc.*** *112*, 6084-6091.

iii) Lipscomb, L. A., Nie, S., Feng, S. and Yu, N.-T. (**1990**) Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser: Gold and Copper Colloids. ***Chem. Phys. Lett.*** 167, 35-40.

iv) Lin, S.-H., Yu, N.-T., Tame, J., Shih, D., Renaud, J.-P., Pagnier, J. and Nagai, K. (**1990**) " The Effect of the Distal Residues on the Vibrational Modes of the Fe-CO Bond in Haemoglobin Studied by Protein Engineering" ***Biochenistry*** *29*, 5562-5566.

v) Tsubaki, M., Ichikawa, Y., Yoshikawa, S., Fujimoto, Y., Yu, N.-T. and Hori, H. (**1990**) "Active Site of Bovine Adrenocortical Cytochrome P-$450_{11\beta}$ Studied by Resonance Raman and Electron Paramagnetic Resonance Spectroscopies: Distinction from Cytochrome P-$450_{scc}$" ***Biochemistry*** *29*, 8805-8810.

vi) Nie, S., and Yu, N.-T. **(1991)** "Surfaced-Enhanced Near-Infrared FT-Raman Scattering of Tetrathiafulvalene Adsorbed on Ag Powder" ***J. Raman Spectrosc.*** (in press).

vii) Bormett, R. W., Asher, S. A., Larkin, P. J., Gustafson, W. G., Ragunathan, N., Freedman, T. B., Nafie, L. A., Balasubramanian, S., Boxer, S., Yu, N.-T., Gersonde, K., Noble, R. W., Springer, B. A. and Sligar, S. G. **(1991)** "Vibrational Circular Dichroism Examination of Azide Ligand Interactions with Hemeprotein Distal Residues" ***J. Amer. Chem. Soc.*** **(submitted).**

viii) Nie, S., Lipscomb, L. A. and Yu, Nai-Teng **(1991)** "Surface-Enhanced Hyper-Raman Spectroscopy" ***Appl. Spectrosc. Rev.*** (in press).